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14. ABSTRACT

Whole-body oxygen consumption is decreased after hemorrhage. Typical methods for increasing oxygen consumption have involved increasing the blood oxygen concentration using enriched oxygen gases, hemoglobins and fluorocarbon compounds; however, clinical trials involving these have not been totally successful. Increasing the oxygen concentration increases its diffusion rate through blood plasma; however, an alternative method would be to increase the diffusion coefficient of oxygen itself. This was shown to be possible using a naturally-occurring compound called crocetin. Crocetin also increased whole-body oxygen consumption in hemorrhaged rats and resulted in an increased survival rate. However, this occurred over a relatively small concentration range. Thus, trans sodium crocetinate (TSC) was developed, which also increases oxygen consumption in rats after hemorrhage and increases survival. TSC has also been shown to increase blood pressure and to reduce the acidosis that forms with hypoxia and to reduce damage to liver and kidney.

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Final Research Report

Recovery from circulatory shock is reported to depend on the restoration of oxygen delivery to the tissues. At the time this research grant began in 1995, we had shown that the carotenoid compound, crocetin, could increase whole-body oxygen consumption (and survival) after moderate shock in rats (1). It was suggested that crocetin had this effect because it increased the diffusivity of oxygen through blood plasma (1). The first experiments performed after the grant started centered on the effect of crocetin during severe shock conditions.

Our previous results had shown that crocetin increases oxygen consumption by an average of 30% after a 40% volume hemorrhage in rats. This increase in oxygen consumption was accompanied by a 100% survival rate for rats treated with crocetin versus a 50% survival rate for the controls. We wanted to use a greater intensity of shock in order to test the effect of crocetin under more severe conditions. Thus, tests were conducted using a 55-60% volume hemorrhage (assuming 60 ml/kg of blood in a rat).

The rats were bled while in an oxygen consumption chamber. The protocol involved anesthetizing the animals with sodium pentobarbital, 50 mg/kg i.p., and exposing the right carotid artery. It was then cannulated using PE-50 polyethylene tubing. The animal was placed in the oxygen consumption chamber and the cannula drawn to the outside of the chamber, through a seal on the lid, and connected to a syringe pump. Oxygen consumption was monitored continuously and the rat not hemorrhaged until its oxygen consumption has returned to a near-normal level. Crocetin was injected, following hemorrhage, through the cannula.

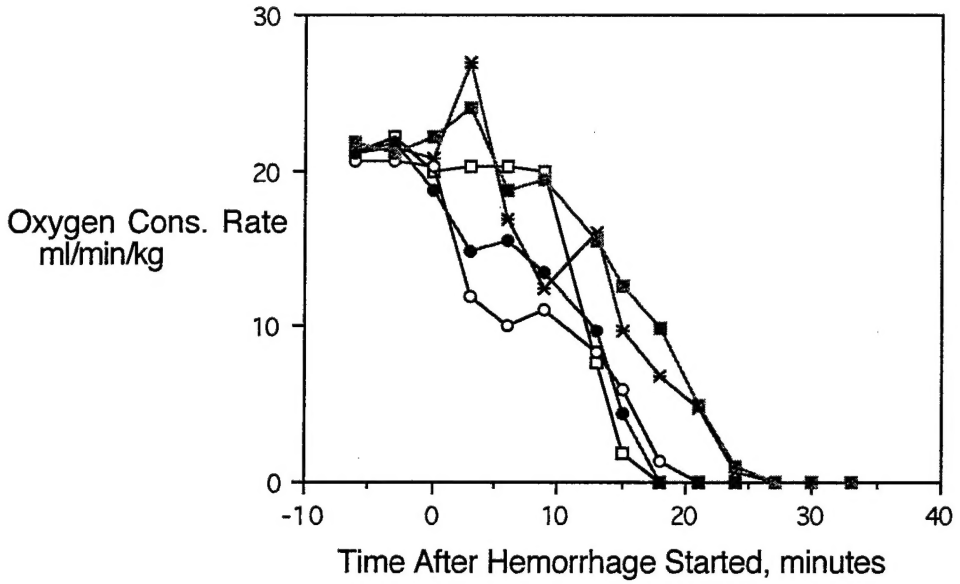
It was found that infusing a solution of crocetin dissolved in normal, isotonic saline caused the whole-body oxygen consumption to increase, after an initial decrease caused by the hemorrhage. But the most important results concerned survival. A rat was considered to have survived if it were breathing at a time of 4.5 hours after the hemorrhage was completed. As shown in the table below, all of the rats receiving crocetin in the infusion fluid survived, while only 40% of the saline-infused or non-treated control rats survived. However, it is felt that the condition of the rat at the survival time is also important. As noted in the table, all of the rats receiving crocetin-supplemented

saline were moving around and seemed to be alert. The response of the rats to noise stimuli was also noted, and all of these rats responded by jumping, moving, *etc.* In contrast, both of the saline-infused rats which survived to this point were very listless and seemed to be "barely alive". They did not respond to noise stimuli, such as a rap on the cage. There were also two rats surviving in the non-treated control group, and one of these was moving and alert while the other one was listless and did not respond to noise.

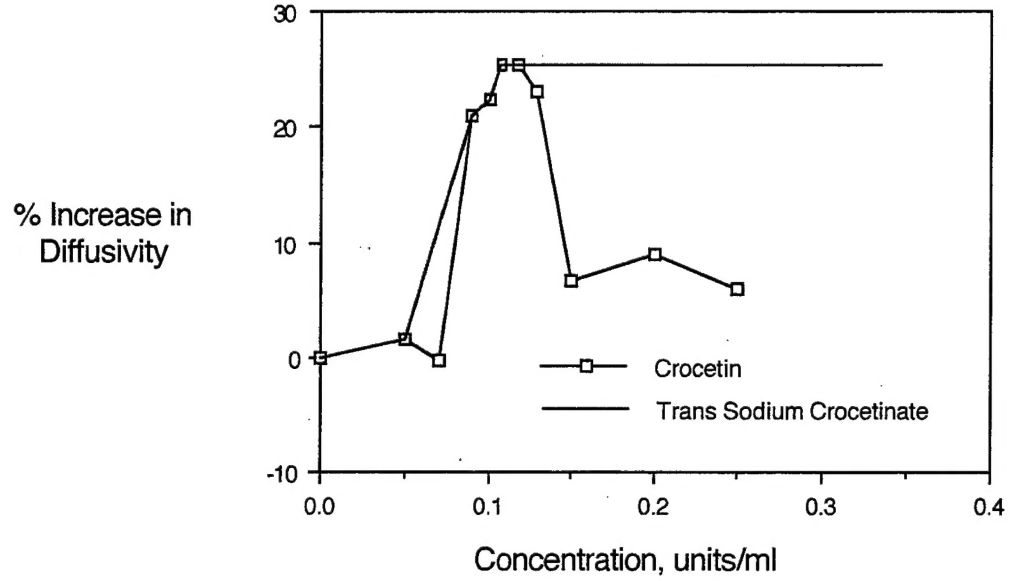
Survival at 4.5 Hours After Hemorrhage

Group	Weight, grams	Survivors	Condition
Controls	324 ± 13	2/5	One was moving and responded to noise. The other was listless and did not respond to noise.
Saline-infused	323 ± 12	2/5	Both were listless and did not respond to noise.
Crocetin-saline infused	326 ± 12	5/5	All were moving and responded to noise.

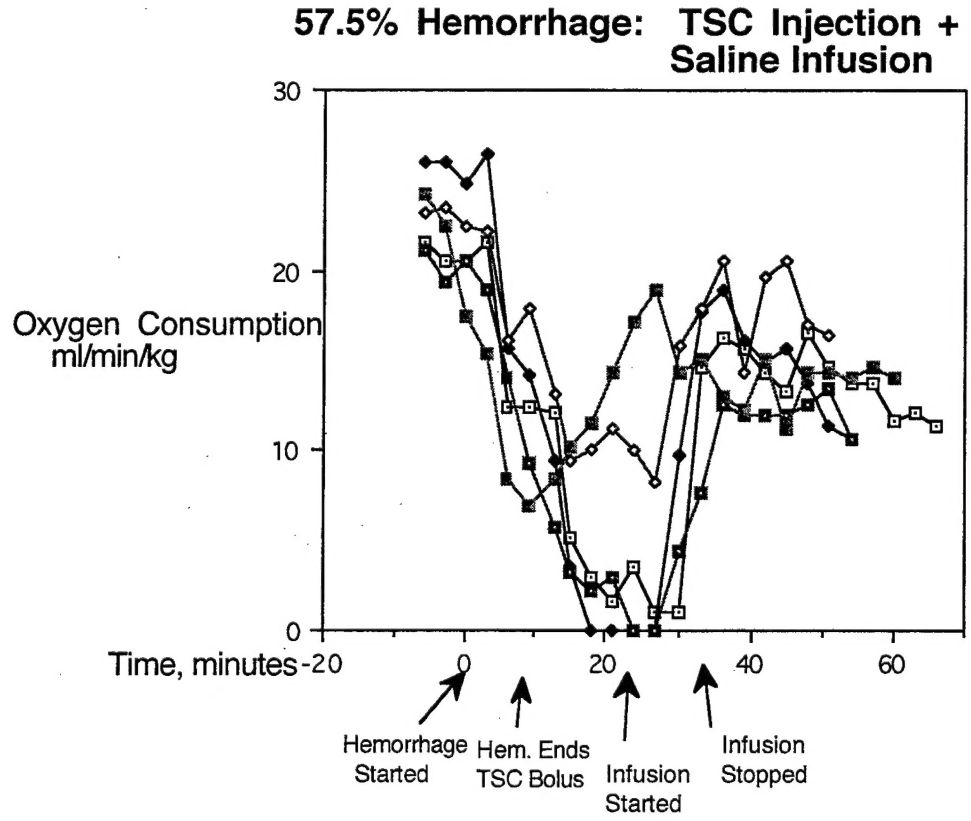
Although these results with crocetin were good, we wanted to extend them and to be able to administer a bolus dosage of crocetin soon after hemorrhage, followed by an isotonic saline resuscitation solution later. This would seem to work better for treating battlefield casualties. We found, however, that crocetin did not work when used like this. The following graph shows that when five rats were injected with crocetin immediately after the hemorrhage ended (with the saline infusion intended to be given 30 minutes later) that their oxygen consumption continued to decline and they all died.



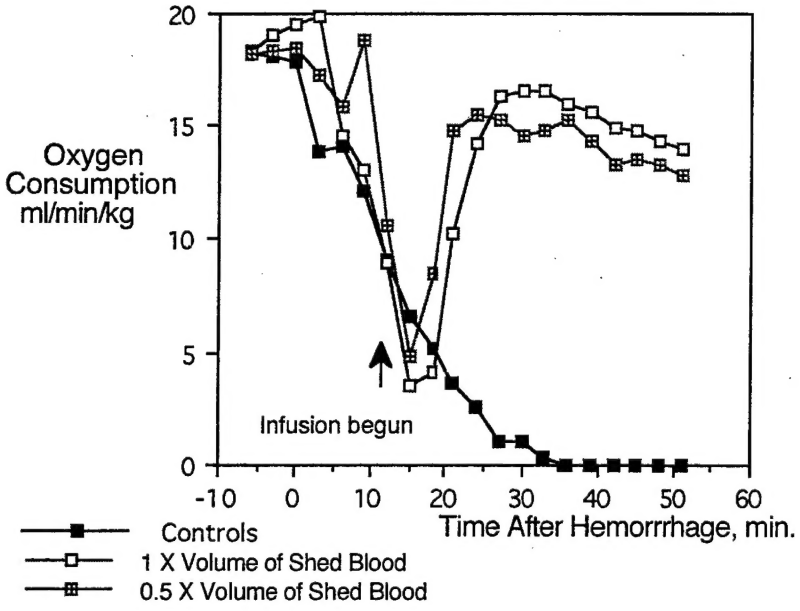
It was felt that the reason for this behavior was due to the fact that crocetin increases the diffusion of oxygen over a relatively small concentration range (1). When given as a bolus injection, the initial concentration must be large because the crocetin clears quickly and it should be present in the blood stream for the life of the study in order to be "active (where the diffusivity is increased). Thus, we began a project in our laboratory to develop a new compound which would increase the diffusivity over a much larger concentration range. Crocetin contains isomers, so we developed a pure trans compound and named it trans sodium crocetinate (TSC). As shown in the next figure, TSC increases the diffusivity over a large concentration range while crocetin does not.



Using TSC instead of crocetin in the previous hemorrhage experiment shows that TSC stabilizes oxygen consumption, which then returns to near-normal values once some fluid (isotonic saline, about 50% of the shed blood volume) has been given to the animal.



These previous data, as well as data for using TSC as an infusion fluid were published (2). For example, it can be seen in the figure below that, if TSC is added to a normal saline infusion fluid. smaller amounts of fluid can be used with the oxygen consumption returning to near-normal.



From this point on, only TSC was used in our studies and most of the subsequent studies involved the effect of bolus injections of TSC on blood pressure, heart rate, blood acidosis and liver enzymes (3,4). First, the effect of a single bolus of TSC with no subsequent saline infusion being given was studied.

The effect of bolus IV injections of TSC on survival is shown in the next table. It is readily apparent that TSC – even at different dosages – increases survival. TSC also has an effect on the blood acidity. Usually in this constant-volume severe hemorrhage model, the blood pH is very low by 15 minutes post-hemorrhage; however, that doesn't happen if TSC is given. That is shown in the second following table.

Survival Following Single Injection After 60% Hemorrhage

Group	N	Survival
Saline Control	5	3 died within 35 minutes 1 alive, but still unconscious at 4 hours 1 alive and alert at 4 hours
TSC (60 mg/kg)	5	5 alive and alert at 4 hours
TSC (180 mg/kg)	5	5 alive and alert at 4 hours

Blood pH

Group	Pre-hemorrhage pH	15 min post-hemorrhage pH
Control	7.488 ± 0.049	7.064 ± 0.177
TSC, 60 mg/kg	7.494 + 0.037	7.293 + 0.061
TSC, 180 mg/kg	7.502 ± 0.038	7.374 ± 0.055

TSC causes the blood pressure to rise when administered after hemorrhage ends, reaching about 80% of its pre-hemorrhaged value after about 30 to 40 minutes after the TSC is given (3). This is not due to an increase in catecholamines, because TSC appears to reduce the epinephrine and norepinephrine produced after hemorrhage (5). It should be noted that TSC also causes a reduction in the tachycardia which results after hemorrhage (3).

In most of the previous studies, TSC was given immediately after the hemorrhage ended. This is, obviously, not practical in many instances, so a study was done in which TSC was given 20 minutes after the hemorrhage ended (this was considered to be the time needed for a first responder to begin treatment). TSC was again able to increase blood pressure and increase survival, but it was only active when given as repeated bolus injections over a period of an hour (4).

A final area studied was the production of cytokines, the inflammatory molecules which have been associated with hemorrhagic shock. For these studies, a different rat hemorrhage model was used. This was a constant-pressure model, where the blood presssure was first lowered to 30 – 40 mm Hg by bleeding and then kept at that level for the next 30 minutes.

The data from these studies show that treatment of hemorrhagic shock with TSC results in lower concentrations of TNF-α in the liver and spleen as well as lower concentrations of IL-10 in the spleen. However, changes in circulating levels of IL-10 were not found. These results are the subject of a manuscript which is currently being written.

Also done in the last phase of this research was a pharmaco-kinetics study. A two-compartment model was fitted to intravenous injection data. It was also found that TSC is absorbed fairly quickly if given via a pulmonary route. This latter method may be better for treating battlefield casualties.

Since TSC sppears to offer promise as a treatment for battlefield casualties, It appears that it is time to scale up this drug for human trials. Diffusion Pharmaceuticals LLC in Charlottesville, VA will be doing the testing needed for filing an IND with the FDA and coordinating the first clinical trials.

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